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PATENT COOPERATION TREATY

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PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P782PC00	**FOR FURTHER ACTION** See Form PCT/IPEA/416	
International application No. PCT/DK2004/000023	International filing date *(day/month/year)* 16.01.2004	Priority date *(day/month/year)* 16.01.2003
International Patent Classification (IPC) or national classification and IPC G01N33/68		
Applicant CARLSBERG A/S		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 13 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 18 sheets, as follows:
      - ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      - ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☒	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand 28.07.2004	Date of completion of this report 29.04.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Weber, P Telephone No. +31 70 340-8982

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/DK2004/000023

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-19, 21-129	as originally filed
20	received on 07.03.2005 with letter of 03.03.2005

Claims, Numbers

1, 2, 4-87	received on 07.03.2005 with letter of 03.03.2005
3	filed with telefax on 12.04.2005

Drawings, Sheets

1/3-3/3	as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☒ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☒ the claims, Nos. 88
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 35-87

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 35-87

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation to restrict or pay additional fees, the applicant has:
   - ☐ restricted the claims.
   - ☐ paid additional fees.
   - ☐ paid additional fees under protest.
   - ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-34 .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-34
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1,3,4,5,6,33
	No:	Claims	2,7-32,34
Industrial applicability (IA)	Yes:	Claims	1-34
	No:	Claims	

2. Citations and explanations (Rule 70.7):

   see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

   and / or

2. Non-written disclosures (Rule 70.9)

   see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

No opinion with regard to novelty, inventive step and industrial applicability will be established for present claims 35-87, because of non-establishment of a search report for former claims 36-88, i.e. present claims 35-87 (see Item IV).

Re Item IV

Lack of unity of invention

The International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: Claims 1-34

Process for identifying specific members of a previously unknown protein-ligand binding pair.

Invention 2: Claims 35-36

Ligands according to formula I and the corresponding ligand-protein binding pairs.

Invention 3: Claims 37-40

Ligands according to formula II and the corresponding ligand-protein binding pairs.

Invention 4: Claims 41-42

Ligands HY1 and HY2 and the corresponding ligand-protein binding pairs.

Invention 5: Claims 43-46

Ligands according to formula IV and the corresponding ligand-protein binding pairs.

Invention 6: Claims 47-48

Ligands comprising or consisting of [SEQ ID NO: 8] and the corresponding ligand-protein binding pairs.

Invention 7: Claims 49-50

Ligands comprising or consisting of [SEQ ID NO: 14] and the corresponding ligand-protein binding pairs.

Invention 8: Claims 51-52

Ligands comprising or consisting of [SEQ ID NO: 11] and the corresponding ligand-protein binding pairs.

Invention 9: Claims 53-54

Ligands comprising or consisting of [SEQ ID NO: 65] and the corresponding ligand-protein binding pairs.

Invention 10: Claims 55-56

Ligands comprising or consisting of [SEQ ID NO: 66] and the corresponding ligand-protein binding pairs.

Invention 11: Claims 57-58

Ligands comprising or consisting of [SEQ ID NO: 67] and the corresponding ligand-protein binding pairs.

Invention 12: Claims 59-60

Ligands comprising or consisting of [SEQ ID NO: 69] and the corresponding ligand-protein binding pairs.

Invention 13: Claims 61-62

Ligands comprising or consisting of [SEQ ID NO: 18] and the corresponding ligand-protein binding pairs.

Invention 14: Claims 63-64

Ligands comprising or consisting of [SEQ ID NO: 56] and the corresponding ligand-protein binding pairs.

Invention 15: Claims 65-66

Ligands comprising or consisting of [SEQ ID NO: 21] and the corresponding ligand-protein binding pairs.

Invention 16: Claims 67-68

Ligands comprising or consisting of [SEQ ID NO: 23] and the corresponding ligand-protein binding pairs.

Invention 17: Claims 69-70

Ligands comprising or consisting of [SEQ ID NO: 27] and the corresponding ligand-protein binding pairs.

Invention 18: Claims 71-72

Ligands comprising or consisting of [SEQ ID NO: 28] and the corresponding ligand-protein binding pairs.

Invention 19: Claims 73-74

Ligands comprising or consisting of [SEQ ID NO: 32] and the corresponding ligand-protein binding pairs.

Invention 20: Claims 75-76

Ligands comprising or consisting of [SEQ ID NO: 35] and the corresponding ligand-protein binding pairs.

Invention 21: Claims 77-78

Ligands comprising or consisting of [SEQ ID NO: 63] and the corresponding ligand-protein binding pairs.

Invention 22: Claims 79-80

Ligands comprising or consisting of [SEQ ID NO: 44] and the corresponding ligand-protein binding pairs.

Invention 23: Claims 81-87

Use of proteins as drug targets in a method to identify one or more drugs for the treatment of a clinical condition.

To fulfil the requirement of unity of invention (Rule 13.1 PCT) there has to be a technical relationship among all of these (groups of) inventions. This relationship has to involve one or more of the same or corresponding special technical features (Rule 13.2 PCT).

The technical feature of process claim 1 resides in the step of observing differential binding of proteins to immobilized ligands in a screening assay in order to specify members of previously unknown protein-ligand binding pairs. Neither the same nor a corresponding special technical feature is present in any of the claimed ligands or the claimed use of proteins. No manufacturing relationship exists between the screening method, the claimed ligands and the use of the proteins. Further, the screening method is neither a method of using the claimed ligands nor a method of using the proteins.

Consequently, there is no single general concept that links the screening method to the claimed ligands and/or the claimed use of the proteins. Thus, unity of invention is lacking (a priori).
The claimed ligands would be regarded as having the same or corresponding technical feature if they had a common property or activity, and shared a significant structural element that is essential to the common property or activity. The claimed ligands neither share a common property nor share a significant structural element, and hence, there is no disclosure of the same or corresponding technical feature.

Consequently, the inventions have been grouped as mentioned above.

No fees were paid for inventions 2-23, hence examination is carried out for invention 1 only.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Reference is made to the following documents:

D1: WO 00/63694 A (UNIV VIRGINIA ; HAYSTEAD TIMOTHY A J (US)) 26 October 2000 (2000-10-26)
D2: WO 00/63701 A (UNIV LELAND STANFORD JUNIOR ; BROWN PATRICK (US); HAAB BRIAN (US)) 26 October 2000 (2000-10-26)
D3: LAM, KIT S. ET AL: "The "One -Bead-One-Compound" Combinatorial Library Method" CHEMICAL REVIEWS, CODEN: CHREAY; ISSN: 0009-2665, vol. 97, no. 2, 1997, pages 411-448, XP002292328 WASHINGTON DC

2 INDEPENDENT CLAIM 1

2.1 The subject-matter of claim 1 is new in the sense of Article 33(2) PCT and involves an inventive step in the sense of Article 33(3) PCT for the following reasons:

2.2 Document D1 is regarded as being the closest prior art to the subject-matter of claim

1, and shows (the references in parentheses applying to this document):

A process for identifying specific members of a previously unknown protein-ligand binding pair, comprising the steps of a) synthesizing a ligand library onto resin beads to form an immobilized ligand library, wherein each bead of the immobilized library comprises one member of the ligand library (implicit in claim 1 and figure 2; explicit on page 11, line 7-19); b) incubating the immobilized ligand library with a protein mixture (claim 1, line 5, step 4 of figure 2); c) detecting an immobilized ligand-protein binding pair from the incubation mixture (implicit in claim 1; explicit in step 7 of figure 2); d) identifying the ligand of the specific ligand-binding pair (claim 1, line 11; step 10 of figure 2); and e) identifying the protein of the ligand-protein binding pair (claim 1, line 10; step 9 of figure 2), wherein the identified ligand and protein are specific members of a previously unknown ligand-protein binding pair (page 5, line 8-10).

The subject-matter of claim 1 differs from this known from D1 in that two differentially labeled protein mixtures are used.

The subject-matter of claim 1 is therefore new (Article 33(2) PCT).

The technical effect of this difference is the detection of ligands, that selectively and/or differentially bind with one set of proteins.

The problem to be solved by the present invention may be regarded as how to detect ligands, that selectively and/or differentially bind with one set of proteins.

The solution to this problem proposed in claim 1 of the present application, i.e. the use of two differentially labeled protein mixtures, is considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

Document D2 discloses in example 1 the use of two differentially labeled protein mixtures for the detection of the binding of immobilized ligands to a plurality of polypeptides for screening and diagnostic purposes. However, document D2 does not relate to libraries (i.e. ensembles of compounds with unknown identity), but to arrays (i.e. ensembles of compounds with known identity). There is no hint for the

person skilled in the art of library screening to transfer the teaching of D2 from array screening to library screening. Consequently, he would not arrive at the present solution without the exercise of inventive skill.

The subject-matter of claim 1 is therefore inventive (Article 33(3) PCT).

3 INDEPENDENT CLAIM 2

3.1 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claim 2 does not involve an inventive step in the sense of Article 33(3) PCT.

3.2 Document D1 is regarded as being the closest prior art to the subject-matter of independent claim 2, and discloses the features already mentioned in Item V 2.

The subject-matter of claims 2 therefore differs from this known from D1 in that the resin beads comprise polyethylene glycol and that at least part of the identification process of the ligand is performed directly on the bead.

However, these features of claim 2 have already been employed for the same purpose, i.e. the identification of bead-bound compounds, in similar library screening process (see document D3, Chapter V, page 436ff). It would be obvious to the person skilled in the art, namely when the same result of improving the identificaton of bead-bound compounds is to be achieved, to apply these features with corresponding effect to a screening process according to document D1, thereby arriving at a screening process according to claim 2.

4 INDEPENDENT CLAIM 3

4.1 The subject-matter of claim 3 is novel in the sense of Article 33(2) PCT and involves an inventive step in the sense of Article 33(3) PCT, because it is neither anticipated by the prior art nor obvious to a person skilled in the art, having regard to the prior art.

5 DEPENDENT CLAIMS 4-6,33

5.1 The subject-matter of claims 4-6,33 is novel in the sense of Article 33(2) PCT and involves an inventive step in the sense of Article 33(3) PCT, because it is neither anticipated by the prior art nor obvious to a person skilled in the art, having regard to the prior art.

6 DEPENDENT CLAIMS 7-32,34

6.1 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 7-32,34 depending on claim 2 does not involve an inventive step in the sense of Article 33(3) PCT.

6.2 Claims 7-32,34 depending on claim 2 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, because all of the features merely represent standard features of one-bead-one-compound libraries and are within the scope of the normal experience and competence of a person skilled in the art.

7 INDUSTRIAL APPLICABILITY (Article 33(4) PCT)

Claims 1-34 meet the requirements of the PCT with respect to the industrial applicability of their subject-matter (Article 33(4) PCT).

8 PRIORITY

The present claims enjoy priority rights from the filing date of the priority document.

(68)

abundance proteins or to isolate a particular class of proteins. General protocols for the extraction of proteins from different organisms are readily available. See, for example, *2-D Proteome Analysis Protocols*, A.J. Link (Ed), 1st Ed, 1999, Humana Press: Totowa)

Detecting

A variety of suitable methods are useful for detecting the ligand-protein binding pairs. For example, where a single protein mixture is used (see Figure 1), the extracted protein may be immediately incubated with the immobilized ligand library, and, after washing, bound protein can be detected directly in the binding complex by the application of a detection molecule to the incubation mixture, such as silver or fluorescent dye that does not interact with the ligand or the solid support. It is however generally preferred that the protein mixture is labeled with a detection probe prior to incubation with the ligand library. Hence, in another embodiment, the mixture of proteins may be labeled with a detection probe, for example, with a fluorescent dye such as Oregon Green 514 (green; See Example 11), N-mehtyl anthranilate (blue; See Example 13), Rhodamine red (red; See Example 11), cyanine dye 2, cyanine dye 3, cyanine dye 5 or other commonly used fluorescent probes. See for example, Richard P. Haugland, "Handbook of Fluorescent Probes and Research Products", 9th Edition, 2002, Molecular Probes Europe BV: Leiden or world wide web (WWW) sites "probes.com" and "amershambiosciences.com/aptrix/upp00919.nsf/Content/DrugScr+CyDye+Fluors+introduction" for a description of cyanine fluorescent dyes. The detection probe may also be a fluorescent protein, such as Green fluorescent proteins or fluorescent mutants thereof. The detection probe can also be a probe that produces chemoluminescence, such as luciferase or aequorin. In these embodiments, after incubation of ligands with proteins, the library is washed and ligand-protein binding complexes will be detected via the label, for example, fluorescence or color. These ligand-protein binding pairs can be immediately isolated using automatic or manual sorting procedures. If the detection probe is a fluorescent probe, then automatic sorting preferably involves the use of a FABS and/or a fluorescence activated beads sorter. The detection probe may furthermore be a compound capable of producing chemiluminescence, such as for example luciferase or aequorin. The detection probe may furthermore be an enzyme capable of catalyzing a detectable reaction, such as for example phosphatase or peroxidase. The detection probe may furthermore be a metal, for example gold. The protein mixture may be labeled with the detection probe by any conventional method depending on the nature of the detection probe.

3. A process for identifying specific members of a previously unknown protein-ligand binding pair, comprising the steps of:

(a) synthesizing a ligand library onto resin beads to form an immobilized ligand library, wherein each bead of the immobilized library comprises one member of the ligand library;

(b) incubating the immobilized ligand library with one or more protein mixture;

(c) detecting an immobilized ligand-protein binding pair from the incubation mixture;

(d) isolating the resin bead comprising the ligand-protein binding pair; and

(e) identifying the ligand ~~and the protein~~ of the ligand-binding pair on the isolated resin bead, wherein at least part of the identification process is performed directly on the bead; and

(f) identifying the protein of the ligand-binding pair on the isolated resin bead, wherein at least part of the identification process is performed directly on the bead;

wherein the identified ligand and protein are specific members of a previously unknown ligand-protein binding pair.